

The Sensitivity of Grass Shrimp, *Palaemonetes pugio*, Embryos to Organophosphate-Induced

Acetylcholinesterase Inhibition

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ABSTRACT

A common inhabitant of salt marshes along the Atlantic and Gulf coasts of North America is the grass shrimp, *Palaemonetes pugio*. Within these salt marshes, grass shrimp fulfill a vital role in energy transfer and ecosystem stability. In South Carolina, agricultural fields are often located adjacent to salt marshes. Since the reproductive period in these animals corresponds to the peak agricultural growing season, grass shrimp at all developmental stages are at risk for pesticide exposure. Organophosphorus (OP) insecticides are commonly applied agricultural chemicals which produce toxicity by inhibiting the nervous system enzyme acetylcholinesterase (AChE). Although adult and larval *P. pugio* have been widely used in toxicity tests to examine various effects of OPs, little research has focused on the effects of these compounds in embryos. The goals of this study were to examine the development of AChE activity in grass shrimp embryos, assess their sensitivity to OP-induced AChE inhibition, and to compare the sensitivity of the embryos to that of larval and adult *P. pugio*. Quantifiable AChE activity first appeared in Stage V embryos and increased as development proceeded. Chlorpyrifos 24-h EC₅₀s were 0.49 µg/L for Stage VI embryos and 0.33 µg/L for Stage VII embryos, while 24-h malathion EC₅₀s were 29.93 µg/L for Stage VI embryos and 55.53 µg/L for Stage VII embryos. Embryonic *P. pugio* were as sensitive to chlorpyrifos and malathion as larval and adult organisms. These findings suggest that *P. pugio* embryo bioassays may provide reasonable predictions as to how these contaminants will affect both larval and adult *P. pugio*.

INTRODUCTION

Grass Shrimp

- Fulfill a vital role in energy transfer and ecosystem stability by accelerating the breakdown of detritus and serving as a prey item for numerous commercially and recreationally important fishes and crustaceans.

- In South Carolina, grass shrimp densities have been estimated at up to 28,000 shrimp/ 50m of stream in summer (Scott et al., 1992).

- Embryos are brooded externally on a female's abdomen for about two weeks prior to hatching and are thus directly exposed to any contaminants that might be present in the estuary.

Organophosphorus (OP) Insecticides

- Commonly used agricultural, vector control, and urban pesticides.

- Toxic action results from the inactivation of an important enzyme of the nervous system, acetylcholinesterase (AChE).

- Chlorpyrifos and malathion are two widely used OPs.

Objectives

- Quantify AChE activity in embryonic grass shrimp.
- Determine the relative sensitivity of Stage VI and Stage VII embryos to both chlorpyrifos and malathion.
- Compare the sensitivity of the embryos to that of larval and adult *P. pugio*.

MATERIALS and METHODS

Test Animals

- Ovigerous *P. pugio* were collected from the western branch of Leadenh Creek, a tidal tributary of the North Edisto River estuary in SC.

- Embryos were staged via a dissecting scope using Wilson's (1985) descriptions of embryonic development (Figure 1).

Baseline AChE Bioassay

- Performed only on Stage IV, V, VI , and VII embryos, since earlier stages did not exhibit AChE activity (Fulton et al., 1994).

- Whole body AChE activity was measured using a modification of Ellman's (1961) original procedure as described by Fulton (1989), Van Dolah et al. (1997), and Key et al. (1998).

24-h EC₅₀ Bioassay

- Performed on Stage VI and Stage VII embryos.

- Embryos were pooled from 3 or 4 females, since 600 embryos were required for each exposure series.

- Standard test conditions of 20 ppt salinity and no aeration were utilized for all 24-h tests (Buikema et al., 1980; Key et al., 1998).

- All tests were conducted in a Revco® environmental chamber at 20°C and a 12-h light: 12-h dark cycle.

- One hundred embryos were exposed in 125 ml Erlenmeyer flasks to each of the five insecticide concentrations and a carrier control (0.1% acetone).

- Nominal exposure concentrations for chlorpyrifos were 0.125, 0.250, 0.500, 1.00, and 2.00 µg/L.

- Nominal exposure concentrations for malathion were 7.50, 15.00, 30.00, 60.00, and 120.00 µg/L.

- Each exposure flask contained 100 ml of media and was placed on a Lab-Line® Orbit shaker set at 180 rpm to prevent fouling of the embryos.

- After 24h of exposure, the embryos were removed from each flask via pipette, wrapped in acetone-rinsed aluminum foil, and stored frozen (-70°C) until analyzed for AChE activity.

Data Analysis

- One-way Analysis of Variance (ANOVAs) were performed to determine if there were significant differences among embryonic developmental stages and insecticide treatments with respect to AChE activity levels.

- Multiple comparison tests, Bonferroni's and Dunnett's, were performed to identify specific group differences.

- Median Effective Concentrations (EC₅₀s) with 95% confidence intervals were obtained using a linear interpolation method: the inhibition concentration approach (Norberg-King, 1993).

RESULTS

Baseline AChE Assay

- Activity levels ranged from 5.42 nmol/mg protein/min for Stage V embryos to 171.59 nmol/mg protein/min for Stage VII embryos (Figure 2).

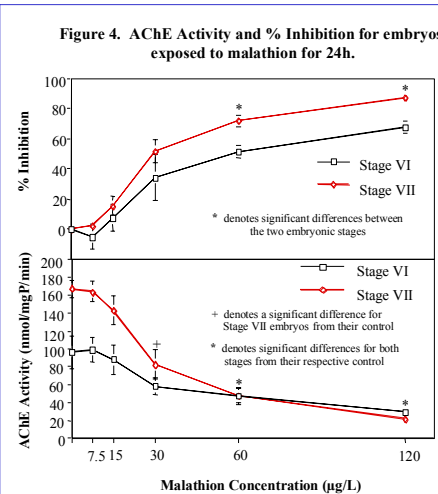
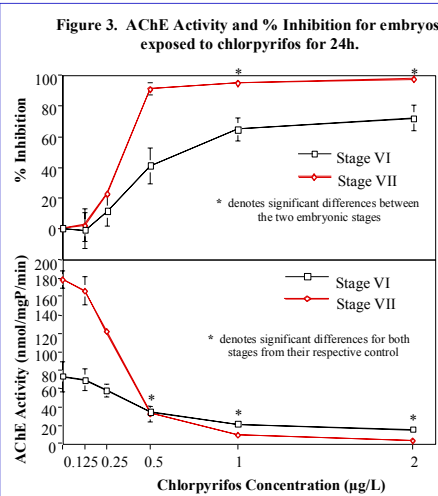
24-h EC₅₀ Bioassay

- AChE activity levels in both Stage VI and Stage VII embryos decreased as the concentration of chlorpyrifos increased (Figure 3).

- The 24-h EC₅₀ values for Stage VI and Stage VII embryos exposed to chlorpyrifos were 0.49 µg/L (95% C.I. = 0.35 - 0.77 µg/L) and 0.36 µg/L (95% C.I. = 0.33 - 0.38 µg/L), respectively (Table 1).

- AChE activity levels in both Stage VI and Stage VII embryos decreased as the concentration of malathion increased (Figure 4).

- The 24-h EC₅₀ values for Stage VI and Stage VII embryos exposed to malathion were 55.53 µg/L (95% C.I. = 22.08 - 80.73 µg/L) and 29.93 µg/L (95% C.I. = 25.22 - 44.22 µg/L), respectively (Table 1).



CONCLUSIONS

- Quantifiable levels of AChE activity first appeared at Stage V of development and significantly increased with each progressive stage of development (Figure 2).

- For both Stage VI and Stage VII embryos, increased insecticide (i.e. chlorpyrifos and malathion) concentrations resulted in decreased AChE activity levels (Figure 3 and Figure 4).

- EC₅₀ values in Stage VI and Stage VII embryos were not significantly different for either of the insecticides (Table 1).

- Stage VII embryos were significantly more sensitive than Stage VI embryos at the two highest exposure concentrations for each insecticide (Figure 3 and Figure 4).
Possible Explanations:
(1). Permeability of embryonic coat (Glas et al., 1996).
(2). Difference in metabolic rate (Howard and Hacker, 1990).
(3). Development of mixed-function oxidase (MFO) system (Ware, 1989).

- A comparison of Stage VI and Stage VII embryos' EC₅₀ values for chlorpyrifos and malathion with laboratory toxicity test results for larvae and adults indicated similar AChE sensitivity for all grass shrimp life history stages (Table 1).

- The distinct advantages (i.e. reduced laboratory space and waste) and equivalent AChE sensitivity provided by the embryo bioassay suggest that this assay may be highly predictive of toxic effects of OP insecticides in grass shrimp populations.

Table 1. 24-h EC₅₀ values for embryonic, larval, and adult *P. pugio* exposed to two OP insecticides.

Life History Stage	Chlorpyrifos	Malathion
Stage VI Embryo	0.49 (0.35-0.77)	55.53 (22.08-80.73)
Stage VII Embryo	0.36 (0.33-0.38)	29.93 (25.22-44.22)
Newly Hatched Larvae	0.56* (0.22-1.17)	7.53* (3.58-15.82)
18-Day Old Larvae	0.28* (0.18-0.44)	24.86* (9.38-65.89)
Adult	1.75* (0.43-7.15)	596.45* (227.69-1560.87)

*Key 1995

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Figure 1. *Palaemonetes pugio* Embryonic Development

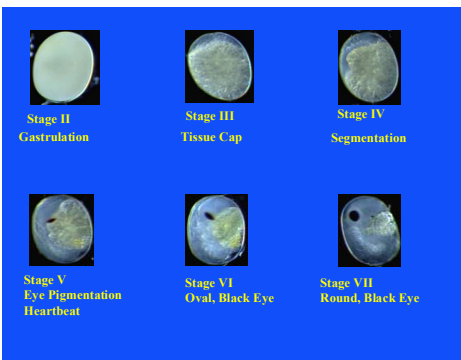


Figure 2. Baseline AChE Activity Levels

